Application Note 01506

Analysis of 15 Azo Dyes Using the Varian 320-MS Triple Quadrupole LC/MS/MS

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Introduction

Azo compounds are formed from the diazotization of aromatic amines. These compounds have been used for decades in the textile, food, and paper industries to dye products a variety of colors. Some azo compounds have been shown to be mutagenic or carcinogenic, and may present a human health risk. As a result, these compounds are prohibited or regulated in food and other products. Therefore, it is important to develop a sensitive, accurate, and selective method to detect and quantify the levels of azo compounds in textile, food and paper products.

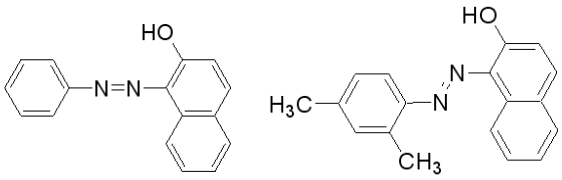

Figure 1. Molecular structure of Sudan I (left) and Sudan II (right) azo dyes.

Currently, the most common analytical methods used for azo compound detection include HPLC with UV or fluorimetric detection.[2] LC/MS/MS provides sensitive and selective detection, with the confidence of LC separation, as well as mass spectral precursor and product ions. In addition, many interferences are eliminated with the use of LC/MS/MS. The LC/MS/MS method outlined herein is sensitive, selective and quantitative, making it a useful tool for regulatory monitoring and quality assessment.

Instrumentation

- Varian 320-MS Triple Quadrupole LC/MS/MS with ESI source
- Varian 212-LC Binary Solvent Delivery Modules
- HTS PAL autosampler from CTC Analytics

Materials & Reagents

Standard azo dyes were purchased from Acros Organics, AppliChem (Darmstadt, Germany), Sigma-Aldrich (which included Sigma, Aldrich, Sigma-Aldrich and Fluka-branded standards) and Spectrum Chemical & Manufacturing Co. All solvents were Optima grade purchased from Fisher Scientific.

Sample Preparation

For each standard, 100 mg of sample was diluted into 1 L of HPLC grade methanol and then diluted to concentrations of 5, 10, 20, 50, 100, and 200 ppb. Orange (II) standard was prepared the same way in HPLC grade water.

HPLC Conditions

Column: Pursuit™ C18 3 µm, 150 x 2.0 mm ID (Varian Part No. A3001150X020)

Solvent A: Water

Solvent B: Acetonitrile

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
00:00	80	20	200
01:00	80	20	200
10:00	0	100	200
25:00	0	100	200
25:01	80	20	200
32:00	80	20	200

Injection Volume: 20 µL

API Conditions

Ionization Mode: ESI (positive/negative)

API Drying Gas: 35 psi at 400 °C

API Nebulizing Gas: 35 psi

Needle: ±4500 V

Shield: ±600 V

Table 1. MS/MS Transitions.

Compound	Q1	Q3 #1	Q3 #2	Capillary Voltage	CID #1	CID #2	Dwell Time(s)
p-Nitroaniline	139.1	92.9	75.9	52	13.0	27.0	0.05
Orange (II)	326.9	155.2	170.8	-68	28.5	22.5	0.05
Rhodamine B	443.2	355.3	399.2	120	45.0	38.0	0.05
Sudan Orange G	215.2	92.9	198.1	44	20.0	11.5	0.05
Fast Garnet GBC	226.1	90.9	121.0	52	14.5	17.5	0.05
Para Red	294.3	127.9	156.0	44	23.0	13.0	0.05
Dimethyl Yellow	226.3	76.9	119.9	52	14.5	27.0	0.05
Sudan Red G	279.1	123.0	248.1	40	13.5	10.0	0.05
Toluidine Red	308.3	127.9	156.0	40	26.5	13.5	0.05
Sudan I	249.0	128.0	156.0	52	22.5	12.5	0.05
Sudan II	277.0	156.0	120.0	48	12.5	10.5	0.05
Sudan III	353.0	156.0	128.0	76	15.5	29.5	0.05
Sudan IV	381.0	224.0	106.0	84	15.5	27.0	0.05
Sudan Red 7B	380.1	169.1	183.1	44	16.5	11.5	0.05
Sudan Black B	457.1	142.0	246.1	88	37.5	20.0	0.05

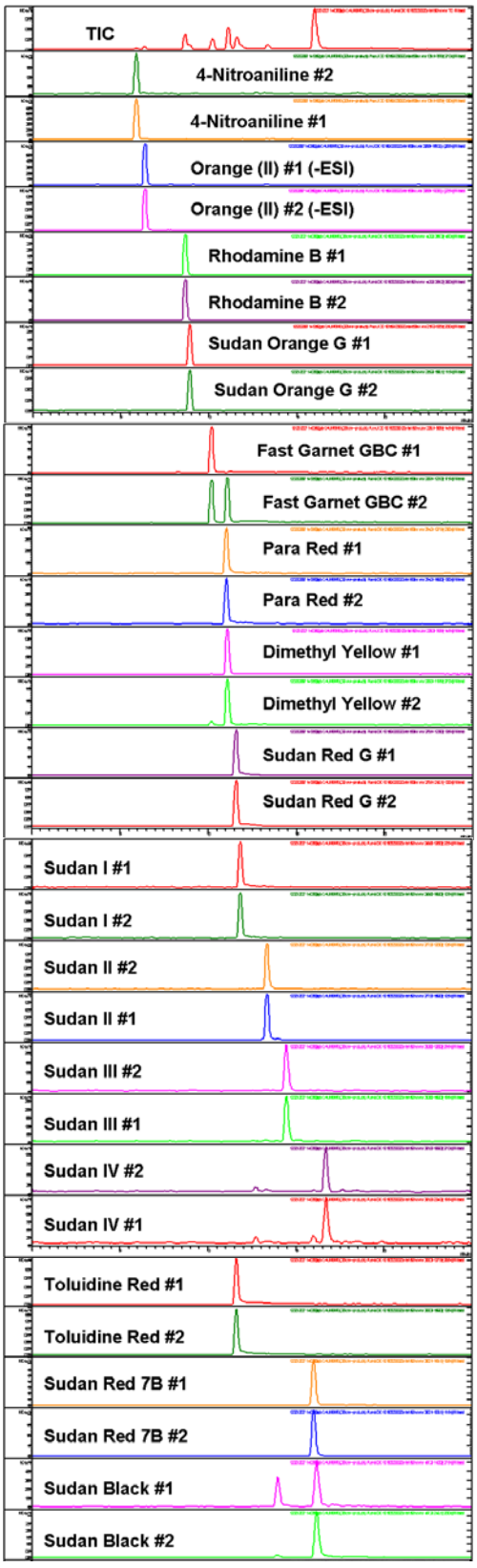

Figure 2. Mass chromatogram for a 50 ppb injection of the azo dyes. The #1 and #2 labels refer to the transitions in Table 1.

Results & Discussion

The MS parameters were optimized for each of the dyes by infusing a 1 ppm standard solution at 20 µL/min while supplying a flow of 50:50 mix of mobile phases A and B at 180 µL/min. This gave a total flow of 200 µL/min of approximately 100 ppb standard mix. Once the MS parameters were optimized for each of the dyes, 20 µL injections were made from 5 ppb to 200 ppb. Figure 2 shows the stacked chromatogram for all 32 transitions of the 15 azo dyes.

Using a single injection for each calibration point, most calibration curves were found to be linear from 5 ppb to 200 ppb. Figure 3 shows the calibration curve for Sudan Black B with %RSD at 8.55% and r^2 value of 0.9999.

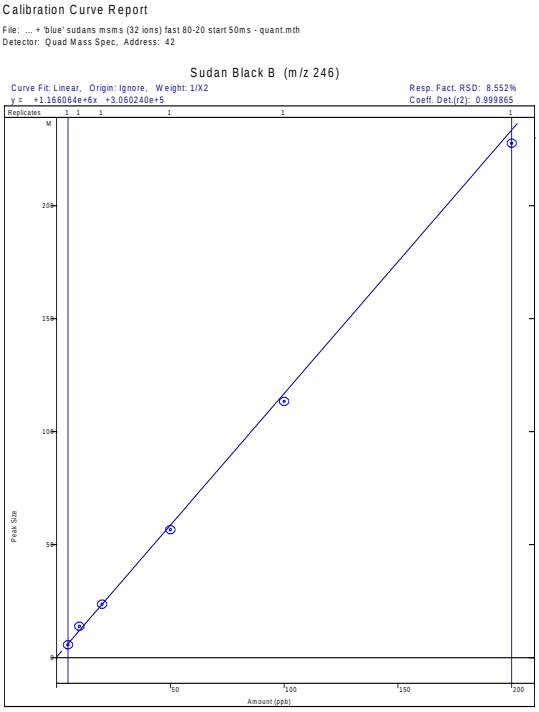

Figure 3. Calibration curve for Sudan Black B from 5 ppb to 200 ppb. RSD is 8.55%, r^2 value is 0.9999.

Conclusion

The Varian 320-MS Triple Quadrupole Mass Spectrometer is able to detect and quantify 15 azo dyes over a wide calibration range. This method is rapid, sensitive and quantitatively accurate, and is performed in a single analytical run with a 20-µL injection.

These data represent typical results.
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